interaction in some cases. Further analysis revealed that the interaction surface, while delocalized, is located within the amino-terminal two-thirds of the c-terminal peptide. Such a delocalized and potentially low-affinity interaction surface is allowed due to the high effective concentration of the c-terminal peptide near the inner vestibule of the pore and likely explains why this region is poorly conserved between species. This type of weak interaction with a tethered gating peptide may be required to maintain high-sensitivity to caspase-dependent activation.

2811-Pos Board B503

Computational Studies of Molecular Permeation through Connexin26 Channels

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Connexin channels are permeable to a wide variety of molecules. Functional studies have shown that channels formed by different connexins have different molecular selectivities, and that the selectivity can strongly depend on factors other than the size of the permeating molecule. This suggests that specific interactions between a molecule and the pore lumen determine whether and how well the molecule can pass through the pore. Molecular Dynamics simulations are widely applied to study permeation of atomic ions through ion-selective channels, and in a few cases, to molecular permeation through transporters and porins. Computational models and strategies must be validated by comparison with experimental results. To this end, we applied both a non-equilibrium simulation method (Steered Molecular Dynamics) and an equilibrium method (Umbrella Sampling Replica Exchange) to explore the free energy profile of two aminopyridyl-labeled saccharides in the connexin26 (Cx26) pore, one of which is permeant and one impermeant, as shown by experiment [1,2]. The system is an MD-equilibrated Cx26 channel, in explicit membrane/solvent, that incorporates key post-translational charge changes and has been shown by Brownian Dynamics to reproduce the electrical conductance characteristics of the native channel [3]. The results show energy profiles that are consistent with experimental results. The permeant has only moderate energy barriers to overcome, while there is a substantial barrier to movement of the impermeant through the pore. Potential sites of interaction within the pore are defined for each molecule. The qualitative correspondence between calculated energy profiles and experimental data for a permeant and a nonpermeant molecule suggests that this system can be used to explore the molecular basis by which connexin channels select among (potential) permeating molecules, and how mutations alter the permeation process.

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2812-Pos Board B504

On the Use of Chemical Modification to Determine Connexin Hemichannel Topology and Function

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Cysteine-scanning mutagenesis combined with thiol reagent modification is a powerful methodology to define pore-lining elements of channels and the changes in structure that accompany channel gating. To help identify the position of the gate of connexin26 (Cx26) hemichannels, using the Xenopus oocyte expression system, we performed cysteine-scanning mutagenesis of several residues within the pore, followed by chemical modification using methanesulfonate (MTS) reagents. Strikingly, we observed that modification with MTS reagents, at different pore lining residues, was reversed within minutes of washout of the reagents. This reversal should not occur unless reducing agents, which can break the disulfide thiol-MTS linkage, have access to the site of modification. We therefore tested whether the connexin pore was allowing cytosolic glutathione, a well-known cytosolic reducing agent, to access the sites. Inhibition of gamma-glutamylcysteine synthetase by buthionine sulphoximine decreased the cytosolic glutathione in Xenopus oocytes and drastically reduced reversibility of MTS modification. In contrast to MTS reagents, maleimide reagents can chemically modify cysteines in a reaction that cannot be reversed by glutathione. As predicted, the maleimide modification did not reverse with washout. Using reconstituted hemichannels in a liposome-based transport-specific fractionation assay, we confirmed that homomeric Cx32, Cx26, Cx30 and heteromeric Cx26/Cx32 and Cx26/Cx30 hemichannels are permeable to glutathione, as others have shown for Cx43 channels. These results suggest that: (a) connexin hemichannels may mediate physiological glutathione release in diverse cell types; (b) maleimide-based modification is the more appropriate approach to perform chemical modification to study structure-function of connexin channels, and other channels and transporters that are permeable to large molecules, such as pannexin channels. Support: GM099490 & GM036044.

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Investigation of Ion Permeation through the Cx26 Hemichannel

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Connexins form the intercellular channels composing gap junctions in vertebrates and providing both the electronic coupling and the exchange of ions and small molecules between adjacent cells. A gap junction channel consists of two opposed hemichannels that were recently shown to be functional when embedded in a nonjunctional membrane. There, the hemichannels mediate release of messengers controlling several important physiological processes including cellular proliferation and tissue remodeling. Moreover, an increasing number of evidence has placed the hemichannels as potential regulators of homeostatic imbalance present in diverse neurodegenerative diseases.

Cx26 is a representative of the gap junction channels with a known structure. Here we resort to molecular modeling methods in order to study process of ion permeation through its hemichannel. Based on obtained trajectories we estimated ion flux and current in a range of voltages applied experimentally for native Cx26 and its two known mutants: D50N and G45E. We have analyzed current-voltage dependencies for the native channel and demonstrated an opposing effect of the two mutations. Finally we studied the process of ion permeation when Ca^{2+} is present in the system. We have shown, for the first time *in silico*, possible modulation of the Cx26 current-voltage dependence by Ca^{2+} ions.

2814-Pos Board B506

The Residues in the First Extracellular Domain Play an Important Role in Transjunctional-Voltage Dependent Gating and Unitary Conductance of Cx50 Gap Junction Channels

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Gap junctions (*GJ*) are intercellular channels that connecting the cytoplasm of neighbor cells. *GJ* channels formed by Cx50 and Cx36 show drastic disparity in their unitary conductance (γ_j) and transjunctional voltage-dependent gating (V_j-gating), but the important underlying molecular domains/residues are not clear. Experimental evidence showed that residues in the first extracellular domain (E1) of Cx50 likely line the *GJ* channel pore and are important factors in determining γ_j and V_j-gating. We aligned the E1 sequence of Cx50 with that of Cx36 and found 10 different residues (4/10 residues involves a change in charge). We generated a chimera Cx50Cx36E1, in which the E1 of Cx50 was replaced by the E1 of Cx36, and 4 point mutations in E1 of Cx50 (where a charge change occurs, i.e. G46E, D51M, E62N and E68R). Dual patch clamp study on the homotypic *GJ* channels formed by the chimera or the point mutants in N2A cell pairs indicate that the Cx50Cx36E1 channel showed little change in the V_j-gating properties, but displayed a significantly reduced main single channel conductance (γ_j). Our studies on the point mutations of Cx50 showed that some of the mutants altered the V_j-gating properties and others changed the γ_j and/or the probabilities in different conducting states. Our study indicates that Cx50 E1 is an important domain in determining the V_j-gating properties and γ_j. Charge changes in different residues in the E1 between Cx50 and Cx36 showed different channel properties likely dictated by either their location in the pore structure or the nature of the mutation.

2815-Pos Board B507

Role of Antibiotic Side Chains in Uptake Through OmpPst1 Channel from Providencia Stuartii

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Penetration through the outer cell wall is the first step for an antibiotic to reach the target site inside the bacteria. In this study, the role of major